Original Article

Evaluation of drug-induced hematotoxicity using novel *in vitro* monkey CFU-GM and BFU-E colony assays

Koichi Goto, Mayumi Goto, Masako Ando-Imaoka, Kiyonori Kai and Kazuhiko Mori

Medicinal Safety Research Laboratories, Daiichi Sankyo Co., Ltd.,
1-16-13 Kita-Kasai, Edogawa-ku, Tokyo 134-8630, Japan

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ABSTRACT — In order to evaluate drug-induced hematotoxicity in monkey cells *in vitro*, colony-forming unit-granulocyte, macrophage (CFU-GM), and burst-forming unit-erythroid (BFU-E) colony assays were established using mononuclear cells in the bone marrow collected from male cynomolgus monkeys. Furthermore, the effects of doxorubicin, chloramphenicol, and linezolid on CFU-GM and BFU-E colony formation were investigated using established monkey CFU-GM and BFU-E colony assays in comparison with those on human CFU-GM and BFU-E colonies acquired from human umbilical cord blood cells. Bone marrow mononuclear cells were collected from the ischial or iliac bone of male cynomolgus monkeys. The cells were subsequently processed by density gradient separation at 1.067, 1.070, or 1.077 g/mL for CFU-GM or 1.077 g/mL for BFU-E, and then cultured in methylcellulose medium for 9 or 13 days, respectively. A sufficient number of CFU-GM colonies were formed from mononuclear cells processed at a density of 1.070 g/mL. Moreover, the number of BFU-E colonies from the cells processed at a density of 1.077 g/mL was sufficient for the colony assay. The number of CFU-GM or BFU-E colonies decreased after treatment with the drugs of interest in a concentration-dependent manner. Compared with human CFU-GM, monkey CFU-GM were more sensitive to chloramphenicol and resistant to doxorubicin, whereas monkey BFU-E were more sensitive to all compounds in comparison to the sensitivity of human BFU-E. In conclusion, monkey CFU-GM and BFU-E colony assays were established and considered useful tools to evaluate the differences in drug-induced hematotoxicity between species.

Key words: Monkey CFU-GM, Monkey BFU-E, Doxorubicin, Chloramphenicol, Linezolid

INTRODUCTION

Hematopoietic organs are injured by many compounds that may affect hematopoietic cell renewal system at different stages, interfering with cell proliferation and differentiation (Amess, 1993). Hematopoiesis is a complex interplay between the intrinsic genetic pathway of blood cells and their environment regulated by cytokines. With the appropriate specific growth factors, hematopoietic stem/progenitor cells can give rise *in vitro* to phenotypically distinct colonies of differentiated cells. Colony formation has been used as an endpoint in *in vitro* studies of xenobiotic myelotoxicity using human, mouse, rat, and canine hematopoietic cells (Parchment, 1998; Parent-Massin, 2001; Pessina *et al.*, 2001; Stephenson *et al.*, 1971; Tepperman *et al.*, 1974). However, to the best of our knowledge, *in vitro* clonogenic assays using mono-

nuclear cells in the bone marrow of monkeys have not yet been reported.

Hematotoxicity is one of the most common adverse effects induced by drugs. In most cases of hematotoxicity, granulocytopenia and agranulocytosis can be caused by many different drugs (Böttiger and Böttiger, 1981; Uetrecht, 1996). Doxorubicin (DOX), an anticancer drug, has been reported to induce neutropenia and thrombocytopenia in humans (Torti *et al.*, 1985). The antibiotic agents chloramphenicol (CAP) and linezolid (LZD) are known to cause aplastic anemia (Malkin *et al.*, 1990) and myelosuppression (Gerson *et al.*, 2002) in humans. However, little information about the preclinical toxicity of these drugs has been reported in monkeys. Monkeys are widely used as non-human primates in the regulatory toxicity testing for drug development. Therefore, it is indispensable for drug development to analyze drug-induced hema-

totoxicity in monkeys and identify differences in hematotoxicity between monkeys and humans.

In the present study, we established monkey colony-forming unit-granulocyte, macrophage (CFU-GM) and burst-forming unit-erythroid (BFU-E) colony assays, and we evaluated the hematotoxic potential of DOX, CAP, and LZD using the colony assays. Difference in CFU-GM and BFU-E colony formation between monkeys and humans was also investigated in these assays.

MATERIALS AND METHODS

Test substances

DOX, CAP, LZD, dimethyl sulfoxide (DMSO), and cremophor EL were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA).

Preparation of test solutions

Test substances were dissolved in DMSO to make 1000 $\times$ stock solutions. These solutions were then diluted with phosphate-buffered saline (Life Technologies Corporation, Carlsbad, CA, USA) mixed with 0.01% cremophor EL to achieve 20 $\times$ stock solutions. As a vehicle control, 0.1% DMSO/0.005% cremophor EL solution was prepared. Final concentrations of each test substance for the CFU-GM colony assay were as follows: 0.5, 1.5, 4.4, 13.3, and 40 nM for DOX; and 10, 30, 100, 300, and 1000 μ M for CAP and LZD. Final concentrations of each test substance for the BFU-E colony assay were as follows: 0.4, 1.6, 6.3, 25, and 100 nM for DOX; and 1, 3, 10, 30, and 10 μ M for CAP and LZD.

Cells

Bone marrow mononuclear cells which were collected from the ischial or iliac bone of a male cynomolgus monkey (5 to 6 years old) and processed by density gradient separation at 1.067, 1.070, or 1.077 g/mL were purchased from Shin Nippon Biomedical Laboratories Ltd. (Kagoshima, Japan) for each assay. Human umbilical cord blood cells were purchased from AllCells, LLC (Alameda, CA, USA).

Preparation of monkey bone marrow mononuclear cells and human umbilical cord blood cells

A cell suspension was prepared by thawing frozen monkey bone marrow mononuclear cells at 37°C. Three hundred microliters of DNase I solution (1 mg/mL, STEMCELL Technologies, Inc., Vancouver, BC, Canada) was added to the cell suspension. Twenty mL of Iscove's modified Dulbecco's medium (IMDM, Life

Technologies Corporation, catalog number: 31980-030) with 2% or 10% fetal bovine serum (FBS, Life Technologies Corporation, catalog number: 10082-147) was carefully added to the monkey cells or human cells, respectively, and then the suspension was centrifuged at 200 $\times$ g for 15 min at room temperature. The resulting supernatant was aspirated carefully and the cells were re-suspended in 20 mL of IMDM with 2% or 10% FBS. This washing process was repeated an additional time. Next, the monkey cells and human cells were re-suspended in 2 mL of IMDM with 2% FBS and IMDM without FBS, respectively. A cell strainer with 100 μ m pores (BD Biosciences, San Jose, CA, USA) was used to obtain a uniform single-cell suspension. Cell concentration of the suspension was measured using a hemocytometer chamber. For monkey cells, IMDM with 2% FBS was added to the cell suspension to obtain a final concentration of 1×10^5 , 2.5×10^5 , 3×10^5 , 6×10^5 , and 1×10^6 cells/mL for the CFU-GM colony assay; 1×10^6 , 2×10^6 , 3×10^6 , and 4×10^6 cells/mL for the BFU-E colony assay; 4×10^5 cells/mL for the CFU-GM colony assay; and 3×10^6 cells/mL for the BFU-E colony assay. For human cells, IMDM was added to make a final concentration of 4×10^5 cells/mL.

Preparation of culture medium

Media and cytokines were purchased from STEMCELL Technologies, Inc. In the present study, MethoCult H4434 and MethoCult H4230 media were selected because MethoCult H4434 was a complete methylcellulose-based medium for the growth of hematopoietic progenitor cells in colony assays of human cells, and MethoCult H4230 was suitable to prepare a similar medium to that reported previously for human CFU-GM colony assay (Pessina *et al.*, 2001) and was used in our in-house studies for human CFU-GM and BFU-E assays. To investigate the method of monkey CFU-GM colony assay, the following methylcellulose-based media were prepared. MethoCult H4434 medium was prepared with 1% antibiotic antimycotic solution (Sigma-Aldrich Co., Ltd.). MethoCult H4230 medium with erythropoietin (EPO) was prepared with 50 ng/mL recombinant human (rh) stem cell factor (SCF), 10 ng/mL rh granulocyte-macrophage-colony stimulating factor (GM-CSF), 10 ng/mL rh granulocyte-colony stimulating factor (G-CSF), 10 ng/mL rh interleukin (IL)-3, 10 ng/mL rh IL-6, 1% antibiotic antimycotic solution, and 3U/mL rh EPO because methylcellulose-based medium supplemented with rh SCF, rh IL-3, rh IL-6, rh G-CSF, rh GM-CSF, and rh EPO was used for colony-forming cell assay using monkey peripheral blood (Carlo-Stella *et al.*, 2004). MethoCult H4230 medium was prepared with 50 ng/mL

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rh SCF, 10 ng/mL rh GM-CSF, 10 ng/mL rh G-CSF, 10 ng/mL rh IL-3, 10 ng/mL rh IL-6, and 1% antibiotic antimycotic solution. For the human CFU-GM colony assay, MethoCult H4230 was prepared with 50 ng/mL rh SCF, 10 ng/mL rh GM-CSF, 10 ng/mL rh G-CSF, 10 ng/mL rh IL-3, 10 ng/mL rh IL-6, and 1% antibiotic antimycotic solution was used. For monkey and human BFU-E colony assays, MethoCult H4230 was prepared with 10 ng/mL rh IL-3, 3U/mL rh EPO, and 1% antibiotic antimycotic solution.

Preparation of assay medium

To investigate the methods of monkey CFU-GM and BFU-E assays, 400 μ L of the cell suspension was added to 3.6 mL of the prepared methylcellulose-based medium in 14-mL BD Falcon polypropylene round-bottom tube (BD Biosciences). For CFU-GM and BFU-E colony assays, 200 μ L of the test solution and 200 μ L of the cell suspension were added to 3.6 mL of the prepared methylcellulose-based culture medium in 14-mL BD Falcon polypropylene round-bottom tube (BD Biosciences). Next, the tubes were mixed vigorously using a vortex mixer and allowed to stand for approximately 5 min until the bubbles dissipated.

Cell culture

One mL of the prepared assay medium with cells was dispensed into 35-mm tissue culture dish (AGC Techno Glass Co., Ltd., Shizuoka, Japan) by using 2.5-mL syringe (Terumo Corporation, Tokyo, Japan). Dispensing procedures were repeated for two more dishes to obtain triplicate samples for each cell concentration or each compound concentration. The medium was distributed evenly across the surface of each dish by gently tilting and rotating the dish to allow the meniscus to attach to the wall of the dish on all sides. After that, one or two dishes were placed into a 100-mm culture dish (AGC Techno Glass Co., Ltd.). An uncovered 35-mm culture dish filled with sterile water was put into each 100-mm dish to maintain humidity. These media samples were incubated at 37°C in air + 5% CO₂ under saturated humidity for 9 days (for CFU-GM colony assay) or 13 days (for BFU-E colony assay).

Counting the CFU-GM and BFU-E colonies

The number of CFU-GM or BFU-E colonies in each 35-mm dish was counted after 9 days or 13 days of incubation using an inverted microscope at 40-fold magnification. A CFU-GM colony was defined as an aggregate containing 50 or more cells. A BFU-E colony was defined as 3 or more closely spaced clusters containing more than

200 well-hemoglobinized red cells.

Statistical analysis

Cell proliferation was expressed as a percentage of growth (inhibition rate) where 100% corresponds to the number of colonies in the vehicle control dishes. The concentrations that inhibited CFU-GM or BFU-E colony formation by 50% (IC₅₀) and by 90% (IC₉₀) were calculated from concentration-response curves in which percent inhibition was plotted against the natural logarithm of the molar concentration of the compound. SAS System Release 9.2 (SAS Institute Inc., Cary, NC, USA) was used for these statistical analyses.

RESULTS

Establishment of monkey CFU-GM colony assay

First, in order to seek an appropriate culture medium for the monkey CFU-GM colony assay, three culture media were used for bone marrow mononuclear cells processed by density gradient separation at 1.077 g/mL. As a result, 1 to 5 CFU-GM colonies/dish were detected using the MethoCult H4434 medium (Figs. 1A and 2A). However, BFU-E colonies or colonies out of recognition were also observed (Figs. 2A and 2B). Two to four CFU-GM colonies/dish were observed using MethoCult H4230 with EPO (Figs. 1A and 2C); however, some BFU-E colonies were also noted (Fig. 2D). No CFU-GM colony was observed using the MethoCult H4230 medium (Fig. 1A); however, colonies out of recognition that were considered BFU-E colonies (lacking any red color) were noted (Figs. 2E and 2F).

Next, the optimal density for the gradient separation of bone marrow mononuclear cells was investigated using the MethoCult H4230 medium. For the bone marrow mononuclear cells processed by density gradient separation at 1.067 g/mL, 0 to 6 CFU-GM colonies/dish were detected at a final concentration of 2.5×10^4 cells/mL; however, no CFU-GM colonies were observed at 1×10^4 cells/mL (data not shown). For the bone marrow mononuclear cells processed by density gradient separation at 1.070 g/mL, 49 to 54 CFU-GM colonies/dish were observed at a final concentration of 1×10^4 cells/mL, 86 to 99 CFU-GM colonies/dish were observed at 3×10^4 cells/mL, and 138 to 166 CFU-GM colonies/dish were observed at 6×10^4 cells/mL (Figs. 1B and 3). However, it was not possible to accurately distinguish each individual CFU-GM colony at 6×10^4 cells/mL because of the high density of colonies. CFU-GM colonies could also not be counted at 1×10^5 cells/mL.

Fig. 1. Number of CFU-GM and BFU-E colonies derived from monkey bone marrow mononuclear cells. Mononuclear cells were obtained from monkey bone marrow processed by density gradient separation at 1.077 g/mL (A and C) or 1.070 g/mL (B). The cells were then cultured in each medium (A) or the MethoCult H4230 medium (B) for 9 days, or the culture medium for BFU-E colony assay (C) for 13 days. A CFU-GM colony was defined as an aggregate containing 50 or more cells. A BFU-E colony was defined as 3 or more closely spaced clusters containing more than 200 well-hemoglobinized red cells. H4434, MethoCult H4434 medium; H4230 with EPO, MethoCult H4230 medium with EPO; H4230, MethoCult H4230 medium. NC, Not counted in cases where there were too many colonies to count.

Establishment of monkey BFU-E colony assay

In the bone marrow mononuclear cells processed by density gradient separation at 1.077 g/mL, 17 to 20, 27 to 30, 28 to 32, and 30 to 41 BFU-E colonies/dish were observed at final concentrations of 1×10^5 , 2×10^5 , 3×10^5 , and 4×10^5 cells/mL, respectively (Figs. 1C and 4). However, discolored red and misshapen colonies were noted (Fig. 4D). Moreover, many cells that did not form BFU-E colonies were also observed (Figs. 4C and 4D).

CFU-GM colony assay

DOX, CAP, and LZD inhibited monkey and human CFU-GM colony formation in a concentration-dependent manner (Fig. 5, Table 1). In the monkey CFU-GM colony assay, the IC_{50} was 4.90 nM for DOX, 9.65 μ M for CAP, and 41.61 μ M for LZD; and the IC_{90} was 72.82 nM for DOX, 252.23 μ M for CAP, and 448.35 μ M for LZD. In the human CFU-GM colony assay, the IC_{50} was 2.75 nM for DOX, 35.41 μ M for CAP, and 39.01 μ M for LZD; and the IC_{90} was 27.84 nM for DOX, 410.64 μ M for CAP, and 479.95 μ M for LZD. The inhibitory effect of three compounds on the CFU-GM colony formation was ranked in the following order: DOX > CAP > LZD for monkey cells and DOX > CAP = LZD for human cells. Furthermore, human CFU-GM was more sensitive to DOX than monkey CFU-GM, but monkey CFU-GM was more sensitive to CAP than human CFU-GM. The effect of LZD on CFU-GM colony formation was thought to be similar

between monkey and human cells.

BFU-E colony assay

DOX, CAP, and LZD inhibited monkey and human BFU-E colony formation in a concentration-dependent manner (Fig. 5, Table 2). In the monkey BFU-E colony assay, the IC_{50} was 4.22 nM for DOX, 4.15 μ M for CAP, and 5.77 μ M for LZD; and the IC_{90} was 48.71 nM for DOX, 16.43 μ M for CAP, and 33.86 μ M for LZD. In the human BFU-E colony assay, the IC_{50} was 15.15 nM for DOX, 11.07 μ M for CAP, and 7.95 μ M for LZD; and the IC_{90} was 246.43 nM for DOX, 128.75 μ M for CAP, and 91.66 μ M for LZD. Based on these data, the inhibitory effect of the three compounds on the BFU-E colony formation was ranked in the following order: DOX > CAP $\geq$ LZD for monkey cells and DOX > LZD $\geq$ CAP for human cells. Furthermore, monkey BFU-E was more sensitive to all three compounds than human BFU-E.

DISCUSSION

In the present study, monkey CFU-GM and BFU-E colony assays were established, and the effects of DOX, CAP, and LZD on the formation of these colonies were compared with the effect on the formation of human CFU-GM and BFU-E colonies.

In the monkey CFU-GM colony assay, the number of CFU-GM colonies was substantially increased by

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Fig. 2. Colonies derived from monkey bone marrow mononuclear cells. Mononuclear cells were obtained from monkey bone marrow processed by density gradient separation at 1.077 g/mL. Next, the cells were cultured in the MethoCult H4434 medium (A and B), MethoCult H4230 medium with EPO (C and D), or MethoCult H4230 medium (E and F) for 9 days. Final cell concentration: 1×10^5 cells/mL. Arrow, CFU-GM colony; Arrow head, BFU-E colony; *, colony out of recognition; **, BFU-E colony due to absence of red color. Original magnification, $\times 40$.

Fig. 3. CFU-GM colonies derived from monkey bone marrow mononuclear cells. Mononuclear cells were obtained from monkey bone marrow processed by density gradient separation at 1.070 g/mL. Next, the cells were cultured in the MethoCult H4230 medium for 9 days. A CFU-GM colony was defined as an aggregate containing 50 or more cells. Final cell concentration: (A) 1×10^4 cells/mL, (B) 3×10^4 cells/mL, (C) 6×10^4 cells/mL, (D) 1×10^5 cells/mL. Original magnification, $\times 40$.

Fig. 4. BFU-E colonies derived from monkey bone marrow mononuclear cells. Mononuclear cells were obtained from monkey bone marrow processed by density gradient separation at 1.077 g/mL. Next, the cells were cultured in the culture medium specific for the BFU-E colony assay for 13 days. A BFU-E colony was defined as 3 or more closely spaced clusters containing more than 200 well-hemoglobinized red cells. Final cell concentration: (A) 1×10^5 cells/mL, (B) 2×10^5 cells/mL, (C) 3×10^5 cells/mL, (D) 4×10^5 cells/mL. Arrow: a discolored red and misshapen colony was observed. Arrow head: Many cells that did not form BFU-E colonies were noted. Original magnification, $\times 40$.

Table 1. Inhibitory concentrations of doxorubicin, chloramphenicol, and linezolid for CFU-GM formation in monkey bone marrow mononuclear cells and human umbilical cord blood cells.

Compound	Monkey CFU-GM		Human CFU-GM	
	IC ₅₀	IC ₉₀	IC ₅₀	IC ₉₀
Doxorubicin (nM)	4.90 (2.96, 8.28) ^a	72.82 (32.83, 299.12)	2.75 (1.91, 3.79)	27.84 (18.11, 50.33)
Chloramphenicol (μM)	9.65 (4.15, 17.03)	252.23 (167.87, 424.31)	35.41 (26.24, 45.80)	410.64 (308.71, 580.02)
Linezolid (μM)	41.61 (32.53, 51.79)	448.35 (346.41, 609.09)	39.01 (29.79, 49.40)	479.95 (362.95, 672.47)

Monkey bone marrow mononuclear cells and human umbilical cord blood cells were cultured in a culture medium treated with doxorubicin, chloramphenicol, or linezolid for 9 days (CFU-GM). The concentrations that inhibited BFU-E colony formation by 50% (IC₅₀) and by 90% (IC₉₀) were calculated from concentration-response curves in which the percent inhibition was plotted against the natural logarithm of the molar concentration of each compound. n = 3. a: 95% confidence interval (Minimum, Maximum).

Table 2. Inhibitory concentrations of doxorubicin, chloramphenicol, and linezolid for BFU-E formation in monkey bone marrow mononuclear cells and human umbilical cord blood cells.

Compound	Monkey BFU-E		Human BFU-E	
	IC ₅₀	IC ₉₀	IC ₅₀	IC ₉₀
Doxorubicin (nM)	4.22 (2.56, 6.64) ^a	48.71 (27.60, 108.51)	15.15 (7.41, 40.47)	246.43 (77.36, 2419.86)
Chloramphenicol (μM)	4.15 (2.73, 6.00)	16.43 (10.83, 30.47)	11.07 (7.03, 17.79)	128.75 (65.02, 386.85)
Linezolid (μM)	5.77 (3.55, 9.50)	33.86 (18.07, 108.85)	7.95 (5.87, 10.64)	91.66 (59.44, 163.66)

Monkey bone marrow mononuclear cells and human umbilical cord blood cells were cultured in a culture medium treated with doxorubicin, chloramphenicol, or linezolid for 13 days (BFU-E). The concentrations that inhibited BFU-E colony formation by 50% (IC₅₀) and by 90% (IC₉₀) were calculated from concentration-response curves in which the percent inhibition was plotted against the natural logarithm of the molar concentration of each compound. n = 3. a: 95% confidence interval (Minimum, Maximum).

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Fig. 5. Inhibitory rate of CFU-GM and BFU-E formation in monkey bone marrow mononuclear cells or human umbilical cord blood cells exposed to doxorubicin, chloramphenicol, or linezolid. Monkey bone marrow mononuclear cells and human umbilical cord blood cells were cultured for 9 days (CFU-GM) or 13 days (BFU-E). (A) monkey CFU-GM, (B) human CFU-GM, (C) monkey BFU-E, (D) human BFU-E. Data are shown as mean $\pm$ S.D. (n = 3).

changing the density from 1.077 g/mL to 1.070 g/mL in the density gradient separation. In human bone marrow cells and peripheral blood cells, it has been reported that CD34⁺ hematopoietic stem/progenitor cells were enriched in low-density gradient separations, even though the total number of cells collected was decreased (Ferrero *et al.*, 1998; Schriber *et al.*, 1995). Taken together, we conclude that the number of monkey CFU-GM colonies increased upon changing the density from 1.077 g/mL to 1.070 g/mL due to the enrichment of CD34⁺ hematopoietic stem/progenitor cells and increased capability to form CFU-GM colonies. In contrast, monkey bone marrow cells processed by density gradient separation at 1.077 g/mL retained the potential to make BFU-E colonies. In human bone marrow cells separated by counterflow centrifugal

elutriation, it has been demonstrated that medium-sized and large CD34⁺ cell populations contained lineage-committed cells which had a potential to differentiate into both CFU-GM and BFU-E, whereas small CD34⁺ cell population included a very primitive progenitor cells possessing capable of yielding both myeloid and lymphoid lineages (Wagner *et al.*, 1995). However, the reason why the monkey cells processed by density gradient separation at 1.077 g/mL did not produce the sufficient number of CFU-GM colonies remains unclear because no data has been reported to show that cell density or cell size of bone marrow cells differed between monkey hematopoietic cells able to form CFU-GM and BFU-E colonies.

In the CFU-GM and BFU-E colony assays, the inhibitory effects of DOX on both CFU-GM and BFU-E colo-

ny formation were strongest among the three compounds. DOX concentration has been reported to be about 10 to 100 nM at 1 hr to 48 hr in human plasma after intravenous treatment with 50 mg/m² of DOX once (Mross *et al.*, 1988). Maximum plasma concentration ($C_{\max}$) of CAP has been shown to be 10 µg/mL (29.6 µM) in humans receiving repeated oral administration at 500 mg for 1 month (Glanzko *et al.*, 1949). It has been demonstrated that $C_{\max}$ of LZD was 18.8 µg/mL (55.6 µM) in humans given repeated (twice daily, every 12 hr) oral administration of 625 mg LZD for 14.5 days (Stalker *et al.*, 2003). Hematotoxicity was observed in humans receiving any of these compounds, suggesting that DOX has the strongest hematotoxic potential among these compounds. This demonstrates that our results correspond to human data *in vivo*.

DOX strongly inhibited the formation of monkey BFU-E and human CFU-GM colonies as compared with that of human BFU-E and monkey CFU-GM, whereas CAP and LZD inhibited BFU-E colony formation for both monkeys and humans. CAP has been reported to decrease hemoglobin concentration but not leukocyte count in monkeys treated intramuscularly with CAP at 350 or 500 mg/kg for 10 days (Hrenoff, 1962). The frequency of leucopenia has been shown to be higher than that of anemia in humans given DOX (Blum and Carter, 1974). It has been reported that CAP induced erythropoietic hypoplasia with reductions of hemoglobin concentration and reticulocyte, and aplastic anemia in humans (Erslev, 1953; Malkin *et al.*, 1990). Moreover, the frequency of anemia caused by LZD has been shown to be higher than that of myelosuppression in humans (Gerson *et al.*, 2002). Based on this information, we considered our results to be consistent with data acquired from *in vivo* studies.

In the CFU-GM assay, the difference between species in susceptibility to the three compounds was as follows: human > monkey for DOX, monkey > human for CAP, and human = monkey for LZD. In the BFU-E colony assay, monkey BFU-E was more susceptible to the three compounds than human BFU-E. Because no data has been reported to compare bone marrow toxicity induced by DOX, CAP, and LZD between monkeys and humans, it is unclear whether or not our results are reproducible *in vivo*. However, our data obtained under the same conditions was considered to reflect the susceptibility of hematopoietic stem and progenitor cells to each compound. Therefore, we conclude that our colony assays can evaluate difference in susceptibility between species to specific compounds in hematopoietic stem and progenitor cells *in vitro*.

In conclusion, a monkey CFU-GM colony assay was established using bone marrow mononuclear cells proc-

essed by density gradient separation at 1.070 g/mL at final cell concentration of 1 to 3 × 10⁴ cells/mL for 9 days. Moreover, a monkey BFU-E colony assay can be performed using bone marrow mononuclear cells processed by density gradient separation at 1.077 g/mL at final cell concentration of 1 to 2 × 10⁵ cells/mL for 13 days. Monkey CFU-GM and BFU-E colony assays established here may serve as a useful tool to evaluate the species-specific effects of drug-induced hematotoxicity in monkeys and humans.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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